- 67 -

We claim:

1. A cell comprising alanine 2,3-aminomutase activity, wherein the cell produces beta-alanine from alpha-alanine.

5

10

- 2. The cell of claim 1, wherein the cell is a transformed cell.
- 3. The cell of claim 2, wherein the cell comprises at least one exogenous nucleic acid molecule, wherein the nucleic acid molecule comprises a nucleic acid sequence that encodes an alanine 2,3-aminomutase.
- 4. The cell of claim 3, wherein the exogenous nucleic acid molecule is a mutated lysine 2,3-aminomutase.
- 15 5. The cell of claim 3, wherein the exogenous nucleic acid molecule is a mutated leucine 2,3-aminomutase.
 - 6. The cell of claim 3, wherein the exogenous nucleic acid molecule is a mutated lysine 5,6-aminomutase.

- 7. The cell of claim 3, wherein the nucleic acid sequence that encodes an alanine 2,3-aminomutase comprises nucleotides 307-1017 of a sequence shown in SEQ ID NO: 20 or nucleotides 55-1026 of a sequence shown in SEQ ID NO: 29.
- 25 8. The cell of claim 7, wherein the nucleic acid comprising nucleotides 307-1017 of SEQ ID NO: 20 or nucleotides 55-1026 of SEQ ID NO: 29 includes one or more substitutions that result in one or more conservative amino acid substitutions.
- The cell of claim 7, wherein the nucleic acid comprising nucleotides 307-1017 of SEQ ID
 NO: 20 or nucleotides 55-1026 of a sequence shown in SEQ ID NO: 29 includes one or more substitutions that result in no more than 10 conservative amino acid substitutions.
 - 10. The cell of claim 3, wherein the nucleic acid sequence that encodes an alanine 2,3-aminomutase comprises a sequence having at least 90% identity to SEQ ID NO: 20 or SEQ ID NO:
- 35 29.

- 11. The cell of claim 10, wherein the nucleic acid sequence that encodes an alanine 2,3-aminomutase comprises a sequence having at least 95% identity to SEQ ID NO: 20 or SEQ ID NO: 29.
- 5 12. The cell of claim 10, wherein the nucleic acid sequence that encodes an alanine 2,3-aminomutase comprises SEQ ID NO: 20 or SEQ ID NO: 29.

10

- 13. The cell of claim 4, wherein the mutated lysine 2,3-aminomutase is a mutated prokaryotic lysine 2,3-aminomutase.
- 14. The cell of claim 13, wherein the mutated prokaryotic lysine 2,3-aminomutase is a mutated Bacillus subtilis, Deinococcus radiodurans, Clostridium subterminale, Porphyromonas gingivalis, or Escherichia coli lysine 2,3-aminomutase.
- 15. The cell of claim 14, wherein the mutated lysine 2,3-aminomutase is a mutated *B. subtilis* lysine 2,3-aminomutase.
 - 16. The cell of claim 15, wherein the mutated *B. subtilis* lysine 2,3-aminomutase comprises an L103M, L103K, L103R, L103E, or L103S substitution.
 - 17. The cell of claim 15, wherein the mutated *B. subtilis* lysine 2,3-aminomutase comprises a L103M, a M136V substitution, a D339H substitution, or any combination thereof.
- 18. The cell of claim 15, wherein the mutated *B. subtilis* lysine 2,3-aminomutase comprises an D339H, D339Q, D339T, or D339N substitution.
 - 19. The cell of claim 14, wherein the mutated lysine 2,3-aminomutase is a mutated *P. gingivalis* lysine 2,3-aminomutase.
- 30 20. The cell of claim 19, wherein the mutated P. gingivalis lysine 2,3-aminomutase comprises an N19Y substitution, an L53P substitution, an H85Q substitution, a D331G substitution, a M342T substitution, or any combination thereof.
- The cell of claim 6, wherein the mutated lysine 5,6-aminomutase is a mutated *C. sticklandii* lysine 5,6-aminomutase.
 - 22. The cell of claim 1, wherein the cell is prokaryotic.

- 23. The cell of claim 22, wherein the prokaryotic cell is a *Lactobacillus*, *Lactococcus*, *Bacillus*, or *Escherichia* cell.
- The cell of claim 22, wherein the prokaryotic cell is an Escherichia coli or Bacillus
 licheniformis cell.
 - 25. The cell of claim 1, wherein the cell is a yeast cell.
 - 26. The cell of claim 1, wherein the cell produces 3-hydropropionic acid (3-HP).

10

27. The cell of claim 26, wherein the cell further comprises:

CoA transferase or CoA synthetase activity;

beta-alanyl-CoA ammonia lyase activity; and

3HP-CoA dehydratase activity.

15

35

- 28. The cell of claim 27, wherein the cell further comprises 3-hydroxypropionyl-CoA hydrolase, and/or 3-hydroxyisobutryl-CoA hydrolase activity.
- 29. The cell of claim 26, wherein the cell further comprises
- 4-aminobutyrate and/or beta-alanine 2-oxoglutarate aminotransferase activity; and
 3-HP dehydrogenase activity or 3-hydroxyisobutyrate dehydrogenase activity.
 - 30. The cell of claim 1, wherein the cell further comprises:

CoA transferase or CoA synthetase activity;

beta-alanyl-CoA ammonia lyase activity;

3-hydroxypropionyl-CoA dehydratase activity;

3-hydroxypropionyl-CoA hydrolase, and/or 3-hydroxyisobutryl-CoA hydrolase activity; and lipase and/or esterase activity.

- The cell of claim 30, wherein the cell produces an ester of 3-HP.
 - 32. The cell of claim 31, wherein the ester of 3-HP is methyl 3-hydroxypropionate, ethyl 3-hydroxypropionate, propyl 3-hydroxypropionate, butyl 3-hydroxypropionate, or 2-ethylhexyl 3-hydroxypropionate.
 - 33. The cell of claim 1, wherein the cell further comprises:

 CoA transferase activity;

beta-alanyl-CoA ammonia lyase activity;

3-hydroxypropionyl-CoA dehydratase activity; an	d
poly hydroxacid synthase activity.	

34. The cell of claim 33, wherein the cell produces polymerized 3-HP.

5

35. The cell of claim 1, wherein the cell further comprises:

CoA transferase activity;

beta-alanyl-CoA ammonia lyase activity; and

poly hydroxacid synthase activity.

10

- 36. The cell of claim 35, wherein the cell produces polymerized acrylate.
- 37. The cell of claim 1, wherein the cell further comprises

CoA transferase activity;

15 beta-alanyl-CoA ammonia lyase activity; and

lipase and/or esterase activity.

- 38. The cell of claim 37, wherein the cell produces an ester of acrylate.
- 20 39. The cell of claim 38, wherein the ester of acrylate is methyl acrylate, ethyl acrylate, propyl acrylate, or butyl acrylate.
 - 40. The cell of claim 1, wherein the cell produces 1,3-propanediol.
- 25 41. The cell of claim 40, wherein the cell further comprises:

CoA transferase or CoA synthetase activity;

beta-alanyl-CoA ammonia lyase activity;

3-hydroxypropionyl-CoA dehydratase activity;

acetylating aldehyde: NAD(+) oxidoreductase activity; and

- 30 alcohol:NAD(+) oxidoreductase activity.
 - 42. The cell of claim 40, wherein the cell further comprises:

CoA transferase activity;

beta-alanyl-CoA ammonia lyase activity;

35 3-hydroxypropionyl-CoA dehydratase activity;

3-hydroxypropionyl-CoA hydrolase, and/or 3-hydroxyisobutryl-CoA hydrolase activity;

aldehyde dehydrogenase (NAD(P)+) activity; and

alcohol dehydrogenase activity.

- 43. The cell of claim 1, wherein the cell produces pantothenate.
- 44. The cell of claim 43, further comprising alpha-ketopantoate hydroxymethyltransferase, alpha-ketopantoate reductase, and pantothenate synthase activity.

45. The cell of claim 43, wherein the cell produces coenzyme A (CoA).

5

10

20

30

35

- 46. The cell of claim 45, further comprising pantothenate kinase, 4'-phosphopantethenoyl-1-cysteine synthetase, 4'-phosphopantothenoylcysteine decarboxylase, ATP:4'-phosphopantetheine adenyltransferase, and dephospho-CoA kinase activity.
- 47. A polypeptide comprising alanine 2,3-aminomutase activity.
- 48. The polypeptide of claim 47, wherein the polypeptide comprises a mutated lysine 2,3aminomutase amino acid sequence.
 - 49. The polypeptide of claim 48, wherein the mutated lysine 2,3-aminomutase amino acid sequence is a mutated *Bacillus subtilis*, *Deinococcus radiodurans*, *Clostridium subterminale*, *Porphyromonas gingivalis* or *Escherichia coli* lysine 2,3-aminomutase.

50. The polypeptide of claim 49, wherein the mutated lysine 2,3-aminomutase amino acid sequence is a mutated *Bacillus subtilis or* mutated *Porphyromonas gingivalis* lysine 2,3-aminomutase.

- The polypeptide of claim 47, wherein the polypeptide comprises amino acids 50-390 of a sequence shown in SEQ ID NO: 21 or amino acids 15-390 of a sequence shown in SEQ ID NO: 30.
 - 52. The polypeptide of claim 47, wherein the polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO: 21 or 30.
 - 53. The polypeptide of claim 52, wherein the polypeptide comprises a sequence having at least 95% sequence identity to SEQ ID NO: 21 or 30.
 - 54. The polypeptide of claim 52, wherein the polypeptide comprises SEQ ID NO: 21 or 30.
 - 55. The polypeptide of claim 52, wherein the polypeptide comprises one or more conservative amino acid substitutions.

- 72 -

- 56. The polypeptide of claim 52, wherein the polypeptide comprises no more than 10 conservative amino acid substitutions.
- 57. An isolated nucleic acid comprising a nucleic acid sequence that encodes the polypeptide of claim 47.
 - 58. The isolated nucleic acid of claim 57 operably linked to a promoter sequence.
- 59. The isolated nucleic acid of claim 57, wherein the nucleic acid comprises nucleotides 307-10 1017 of SEQ ID NO: 20 or nucleotides 55-1026 of SEQ ID NO: 29.
 - 60. The isolated nucleic acid of claim 57, wherein the nucleic acid comprises a sequence having at least 90% identity to SEQ ID NO: 20 or SEQ ID NO: 29.
- 15 61. The isolated nucleic acid of claim 57, wherein the nucleic acid comprises a sequence having at least 95% identity to SEQ ID NO: 20 or SEQ ID NO: 29.
 - 62. The isolated nucleic acid of claim 60, wherein the nucleic acid sequence includes one or more substitutions which results in one or more conservative amino acid substitutions.
 - 63. The isolated nucleic acid of claim 60, wherein the nucleic acid sequence includes one or more substitutions which results in no more than 10 conservative amino acid substitutions.
- 64. The isolated nucleic acid of claim 61, wherein the nucleic acid comprises SEQ ID NO: 20 or 25.
 - 65. A vector comprising the isolated nucleic acid of claim 57.

20

- 66. A recombinant nucleic acid comprising the isolated nucleic acid of claim 57.
- 67. A cell transformed with the recombinant nucleic acid of claim 66.
- 68. A non-human transgenic mammal comprising the recombinant nucleic acid of claim 57.
- 35 69. A transformed cell comprising at least one exogenous nucleic acid molecule, wherein the at least one exogenous nucleic acid molecule comprises a nucleic acid sequence that encodes the polypeptide of claim 47.

- 73 -

- 70. The transformed cell of claim 69, wherein the cell produces beta-alanine from alpha-alanine.
- 71. The cell of claim 70, wherein the cell produces 3-HP.
- 5 72. The cell of claim 71, wherein the cell produces 1,3-propanediol.
 - 73. The cell of claim 70, wherein the cell produces pantothenate.
 - 74. The cell of claim 73, wherein the cell produces CoA.

1Ò

15

- 75. A specific binding agent that specifically binds to the polypeptide of claim 47.
- 76. A method of producing a polypeptide comprising alanine 2,3-aminomutase activity, comprising culturing the cell of claim 67 under conditions that allow the cell to produce the polypeptide comprising alanine 2,3-aminomutase activity.
- 77. A method for making beta-alanine from alpha-alanine, comprising culturing the cell of claim 1 under conditions that allow the cell to make beta-alanine from alpha-alanine.
- 78. The method of claim 77, wherein the cell comprises at least one exogenous nucleic acid molecule that encodes an alanine 2,3-aminomutase, wherein the alanine 2,3-aminomutase is capable of producing the beta-alanine from the alpha-alanine.
 - 79. The method of claim 78, wherein the cell is a prokaryotic cell.

25

- 80. The method of claim 78, wherein the cell is a yeast, *Lactobacillus*, *Lactococcus*, *Bacillus*, or *Escherichia* cell.
- 81. The method of claim 78, wherein the cell comprises a functional deletion of panD.

- 82. A method of identifying a cell comprising alanine 2,3-aminomutase activity, comprising: culturing a cell functionally deleted for *panD* in media comprising no beta-alanine and no pantothenate; and
- identifying a cell which is capable of growing in the media, wherein growth of the cell indicates that the cell is producing beta-alanine from alpha-alanine, which indicates the cell comprises alanine 2,3-aminomutase activity, and wherein absence of growth of the cell indicates that the cell is not producing beta-alanine from alpha-alanine, which indicates the cell does not comprise alanine 2,3-aminomutase activity.

- 74 -

- 83. The method of claim 82, further comprising transfecting the cell with one or more mutated lysine 2,3-aminomutases, prior to the step of culturing the cell.
- 84. The method of claim 83, wherein the one or more mutated prokaryotic lysine 2,3aminomutase is a mutated Bacillus subtilis, Deinococcus radiodurans, Clostridium subterminale,
 Aquifex aeolicus, Haemophilus influenza, Escherichia coli and/or Porphyromonas gingivalis lysine
 2,3-aminomutase.
- 85. The method of claim 83, wherein the one or more mutated lysine 2,3-aminomutases is a mutated *B. subtilis* lysine 2,3-aminomutase.
 - 86. The method of claim 83, further comprising identifying a mutation in the one or more mutated lysine 2,3-aminomutases which confer the alanine 2,3-aminomutase activity to the cell, following identifying a cell which grows in the media.

87. The method of claim 86, wherein identifying the mutation in the one or more mutated lysine 2,3-aminomutases comprises sequencing the one or more mutated lysine 2,3-aminomutases.

88. The method of claim 82, wherein the cell is a prokaryotic cell.

20

15

- 89. A method for making 3-HP, comprising culturing the cell of claim 1 under conditions wherein the cell produces the 3-HP.
- 90. The method of claim 89, wherein the cell comprises at least one exogenous nucleic acid that encodes an alanine 2,3-aminomutase such that the 3-HP is produced from beta-alanine, wherein the alanine 2,3-aminomutase produces beta-alanine from alpha-alanine.
 - 91. The method of claim 89, wherein the cell further comprises:

CoA transferase or CoA synthetase activity;

30 beta-alanyl-CoA ammonia lyase activity;

3-HP-CoA dehydratase activity; and

- 3-hydroxypropionyl-CoA hydrolase, and/or 3-hydroxyisobutryl-CoA hydrolase activity
- 92. The method of claim 89, wherein the cell further comprises:
- 4-aminobutyrate and/or beta alanine-2-oxoglutarate aminotransferase activity; and 3-HP dehydrogenase and/or 3-hydroxybutyrate dehydrogenase activity.

PCT/US03/01635

WO 03/062173

- 75 -

- 93. A method for making 1,3-propanediol, comprising culturing the cell of claim 40 under conditions wherein the cell produces the 1,3-propanediol.
- 94. A method for making pantothenate, comprising culturing the cell of claim 43 under 5 conditions wherein the cell produces the pantothenate.
 - 95. A method for making CoA comprising culturing the cell of claim 45 under conditions wherein the cell produces the CoA.
- 10 96. A method for making 3-HP, comprising: purifying beta-alanine from the cell of claim 1;

contacting the beta-alanine with a polypeptide comprising CoA transferase activity to form beta-alanyl-CoA;

contacting the beta-alanine CoA with a polypeptide comprising beta-alanyl-CoA ammonia 15 lyase activity to form acrylyl-CoA;

contacting the acrylyl-CoA with a polypeptide comprising 3HP-CoA dehydratase activity to form 3-HP-CoA; and

contacting 3-HP-CoA with a polypeptide comprising CoA transferase activity, 3hydroxypropionyl-CoA hydrolase, and/or 3-hydroxyisobutryl-CoA hydrolase activity to make 3-HP.

20

25

97. A method for making 3-HP, comprising: purifying beta-alanine from the cell of claim 1;

contacting the beta-alanine with a polypeptide comprising 4-aminobutyrate aminotransferase and/or beta-alanine - 2-oxoglutarate aminotransferase activity to form malonic semialdehyde; and

- contacting the malonic semialdehyde with a polypeptide comprising 3-HP dehydrogenase and/or 3-hydroxyisobutyrate dehydrogenase activity to make 3-HP.
- 98.. A method for making 3-HP, comprising:

transfecting the cell of claim 1, with a nucleic acid encoding a polypeptide comprising CoA 30 transferase activity, with a nucleic acid encoding a polypeptide comprising beta-alanyl-CoA ammonia lyase activity, and with a nucleic acid encoding a polypeptide comprising CoA transferase activity, 3hydroxypropionyl-CoA hydrolase, and/or 3-hydroxyisobutryl-CoA hydrolase activity; and culturing the transfected cell to allow the transfected cell to make 3-HP.

35 .99. A method for making 3-HP, comprising:

> transfecting the cell of claim 1, with a nucleic acid encoding a polypeptide comprising 4aminobutyrate aminotransferase and/or beta-alanine-2-oxoglutarate aminotransferase activity and with a nucleic acid encoding a polypeptide comprising 3-HP dehydrogenase and/or 3-

- 76 -

hydroxyisobutyrate dehydrogenase activity; and culturing the transfected cell to allow the transfected cell to make 3-HP.

5

102.

- 100. A method for making 1,3-propanediol from 3-HP, comprising:
 making 3-HP using the method of claim 97;
 contacting the 3-HP with a polypeptide comprising acetylating aldehyde:NAD(+)
 oxidoreductase activity and a polypeptide comprising alcohol:NAD(+) oxidoreductase activity.
- 101. A method for making 1,3-propanediol, comprising:
 10 transfecting the cell of claim 1 with a nucleic acid encoding a polypeptide comprising CoA transferase or CoA synthetase activity; with a nucleic acid encoding a polypeptide comprising beta-alanyl-CoA ammonia lyase activity; a nucleic acid encoding a polypeptide comprising, 3-hydroxypropionyl-CoA hydrolase, and/or 3-hydroxyisobutryl-CoA hydrolase activity; a nucleic acid encoding a polypeptide comprising acetylating aldehyde:NAD(+) oxidoreductase activity; and a
 15 nucleic acid encoding a polypeptide comprising alcohol:NAD(+) oxidoreductase activity; and culturing the transfected cell to allow the transfected cell to make 1,3-propanediol.
- transfecting the cell of claim 1 with a nucleic acid encoding a polypeptide comprising CoA

 transferase or CoA synthetase activity; with a nucleic acid encoding a polypeptide comprising betaalanyl-CoA ammonia lyase activity; with a nucleic acid encoding a polypeptide comprising 3hydroxypropionyl-CoA dehydratase activity; with a nucleic acid encoding a polypeptide comprising
 3-hydroxypropionyl-CoA hydrolase, and/or 3-hydroxyisobutryl-CoA hydrolase activity; with a
 nucleic acid encoding a polypeptide comprising aldehyde dehydrogenase (NAD(P)+) activity; with a
 nucleic acid encoding a polypeptide comprising alcohol dehydrogenase activity and

A method for making 1,3-propanediol, comprising:

- culturing the transfected cell to allow the transfected cell to make 1,3-propanediol.
- 103. A method for making pantothenate, comprising:

 purifying beta-alanine from the cell of claim 1; and

 contacting the beta-alanine with alpha-ketopantoate hydroxymethyltransferase, alpha-ketopantoate reductase, and pantothenate synthase to make pantothenate.
- 104. A method for making pantothenate, comprising:
 transfecting the cell of claim 1 with a nucleic acid encoding a polypeptide comprising alpha ketopantoate hydroxymethyltransferase activity, a nucleic acid encoding a polypeptide comprising alpha-ketopantoate reductase activity, and a nucleic acid encoding a polypeptide comprising pantothenate synthase activity; and

culturing the transfected cell to allow the transfected cell to make pantothenate.

- 77 -

- 105. The cell of claim 1, wherein the cell is a plant cell.
- 106. A plant comprising the cell of claim 104.
- 5 106. A transgenic plant comprising the recombinant nucleic acid of claim 57.